Remarks

Claims 1, 6, 7, 10 and 14-21 are pending (please note that claims 32 and 33 had been canceled in the previous Response mailed on July 29, 2002). Claims 10 and 14-18 were amended to provide proper antecedent basis. Claim 7 was amended to properly read as a "Markush" claim. Claim 1 was amended to recite "the improvement comprising expressing in the *E. coli* a D-specific enoyl-CoA hydratase and ß-hydroxyacyl-ACP-coenzymeA transferase, and providing feedstocks for the transgenic *E. coli*, wherein the enzymes are expressed in a sufficient amount to produce polyhydroxybutyrate-co- polyhydroxyhexanoate." Claims 8, 9, 11-13, 22-27 and 31 have been canceled. The cancellation of the preceding-identified claims was made to facilitate prosecution. It is the applicant's understanding that the pending claims still encompass the subject matter deleted/canceled.

The present invention is directed to the production of polyhydroxybutyrate-co-polyhydroxyhexanoate. The methods are limited to production of PHAs containing 3HH in *E. coli*. *E. coli* do not normally produce PHAs and have not previously been described to produce 3HH copolymers. The claimed methods rely upon the activities of polymerase/synthase enzymes that accept 3-hydroxyhexanoyl-CoA type molecules and more preferably 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl-CoA type molecules as substrates.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1, 6-27 and 31 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiner stated that Examples 2 and 4, as provided in the specification, "show that the interactions between the claimed enzymes are not predictable." Again, it must be noted that interactions between enzymes, *per se*, should not be at issue, but rather interactions between *substrates and enzymes*. One of ordinary skill in the art will readily agree with the notion that a substrate in the presence of its cognate, active enzyme, will be readily converted into product (i.e. the substrate for another enzyme). Again, for example, a phbA thiolase gene encoding an enzyme that converts butyryl-CoA (substrate) and acetyl CoA (substrate) to beta-ketohexanoyl-CoA (product) does not represent a "novel interaction" between substrate and enzyme. *The claimed enzymes do not physically interact with one another.* However, substrates and enzymes do interact.

Furthermore, based upon the written description, one of ordinary skill in the art will appreciate that the presence, or production, of end-product (i.e. polyhydroxybutyrate-co-polyhydroxyhexanoate) is easily measured and characterized using methods well known in the art (for example, see page 18, line 29, to page 19, line 10). The presence of end-product dictates whether or not the overall process of providing proper substrate for enzyme catalysis at each step in a pathway is successful. As the Examiner has properly pointed out, Example 2 provides a

transgenic *E. coli* harboring pMBXc12J12 (*A. caviae* PHB polymerase gene) and pSU18-AB1 (*R. eutropha phbAB* genes). The *product* polymer composition was based upon the substrate feed glucose and butyrate. The discovery that a broad substrate range reductase and a polymerase that accepts more than one type of substrate could be used in a single pathway dedicated to the synthesis of polyhydroxybutyrate-co-polyhydroxyhexanoate, ***was a completely unexpected result*** (resulting in PHBH copolymer containing 1.0% HH co-monomer; please see Examples). Again, this pathway is predicated upon efficient *substrate/enzyme* interactions and reactions. As such, the Applicants submit that the emphasis here is on substrates and products. However, in view of the Examiner's concerns directed to the "redirection of secondary metabolites toward the production of PBPH" (see page 5 of Office Action mailed on December 9, 2002; and the references cited in the Office Action mailed on March 28, 2002), the Applicant's submit that the characterization of secondary metabolic pathways, and butyryl Co-A dehydrogenase (BCD) activity in plants and extracts, is completely irrelevant to the claimed methods within an intact *E. coli* cell system. Levels of predictability obviously vary across different systems with regard to the amount of experimentation required to produce the desired outcome. The cited references do not apply to the state of the art (genetics of an *intact bacterial system*) and therefore should not be considered as factors for a determination of the state of the prior art. Furthermore, Example 2 clearly shows the successful production of polyhydroxybutyrate-co-polyhydroxyhexanoate in intact *E. coli* cells. The applicants respectfully request further clarification as to why one of ordinary skill in the art would be

preoccupied with secondary metabolic pathways in a system clearly shown to work and produce polyhydroxybutyrate-co-polyhydroxyhexanoate using a broad range reductase and a polymerase that accepts 3-hydroxyahexanoyl CoA and 3-hydroxybutyryl-CoA as substrate (for example, see Example 2)? The consideration given to secondary metabolic pathways by the Examiner is clearly undue and unnecessary.

Claims 1, 6-27 and 31 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiner asserted that a precise description of the structure of the gene is required to indicate possession of the claimed invention. The Examples illustrate that the applicants had possession of a method for the biological production of polyhydroxyalkanoate containing 3-hydroxyhexanoate comprising growing a transgenic *E.coli* having the claimed transgenes at the time of filing.

As stated in M.P.E.P. § 2173.05(t), which describes the standard to be applied to compounds and compositions, "a compound of unknown structure may be claimed by a combination of physical and chemical characteristics." See *Ex parte Brian*, 118 USPQ 242 (Bd. App. 1958). M.P.E.P. § 2173.05(t) further states that "a compound may also be claimed in terms

of the process by which it is made without raising an issue of indefiniteness." It is important to note that, in addition to those nucleic acid sequences defined as specific phbA, phbB, PHB, phbC, and phaJ genes in the specification, the primer/oligonucleotide sequences used to hybridize to, and isolate, ***those sequences can be used to isolate other genes encoding the enzymes of the claimed invention***. This is routine to those skilled in the art. Methods include "colony blotting using the corresponding PCR products as probes" (see page 12, lines 13-15), colony blot hybridization using full length genes as probes (see, for example, page 20, lines 13-16), Southern blotting, for example, wherein "DNA fragments containing the *phbB* and *phbC* genes from *N. salmonicolor* were identified in genomic digests by Southern blotting using the corresponding PCR products as probes" (see page 12, lines 11-13), and PCR isolation/amplification, for example, wherein primers that are based on the nucleotide sequence of the *phaC* gene from *Rhodococcus ruber* and conserved regions in the N- and C-terminal ends of known acetoacetyl CoA dehydrogenases are used to isolate the corresponding PHB biosynthetic enzymes form *N. salmonicolor* (see page 12, lines 4-11). Therefore, the methods in which one of ordinary skill in the art would use to isolate the claimed genes lie at the very heart of defining the structural nature of each. The structural features common to the members of the claimed genus of *pha* genes have *already* been determined based upon the hydrogen bonding arrangement of the primers/oligos used to isolate such sequences. The structures of the claimed genes are clearly limited based, in part, on the requirement for them to be complementary to the primers/oligos disclosed.

The cited enzymes, as claimed, are known and well characterized. Their sequences are available in public databases and their sources are cited in the specification (examples provided the enclosed National Center for Biotechnology Information sequence listings; phbB from *Asospirillum brasilense*; phbA, phbB, phbC from *Pseudomonas putida*; phbC from *Rhodobacter sphaeroides*; phbC from *Azorhizobium caulinodans*; phaC from *Pseudomonas sp.*; and phbC from *A. eutrophus*). The amount of direction or guidance presented in the specification, in combination with the state of the art at the time of filing, clearly provides an enabling disclosure for, *inter alia*, obtaining any of the requisite genes. As described in the specification, genes may be isolated using a number of techniques, including the use of oligonucleotide primers designed to be complementary to the known sequence (and/or degenerate primers) in conjunction with PCR. Once isolated, construction of gene expression cassettes and transformative plasmids, as described in the specification, are easily produced. Plasmids are introduced *via* known methods, such as transformation.

The complementary nature of the claimed gene sequences to known primers/oligos distinguishes the claimed genes from others. Gene sequences that bind the primers/oligos have the requisite correct charge and spatial orientation of the potential hydrogen bond donors and acceptors to be specific for presentation and binding to the primer/oligo. Therefore the structural features common to the claimed genes, as defined in the specification by their complementarity to PCR oligos (containing the requisite hydrogen bonding acceptor and donor sites), are clearly described. In order for any nucleic acid sequence to have a structure that is complementary to

any oligonucleotide, hydrogen bond donor and acceptor sites must be properly arranged for hybridization to occur. To this end, each primer/oligo describes, defines (in part) and limits the structure of the claimed genus of genes encoding the claimed biosynthetic enzymes.

The specification also discloses other relevant information and identifying characteristics sufficient to describe the claimed invention. One of skill in the art would be able to predict the structure of the claimed complementary/hybridizing genes from the recitation of their functions. As had been previously submitted, each of the genes harbored by the claimed transgenic organism have defined structural and functional characteristics. Known substrates, for each of the claimed enzymes, dictate structural features within the "pockets" of each enzyme to which they bind. The encoded gene products (each of the claimed, encoded enzymes) recognize known substrates and produce known products. Such functional characteristics are predicated on the structural features of each.

Moreover, the relationship between these features of the claimed genes, as well as their function, completely detail to those skilled in the art that applicants possessed the claimed genus at the time of filing this application, and enabled those skilled in the art to know what does, and does not, fall within the scope of the claims.

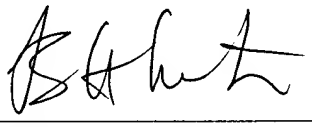
Rejection Under 35 U.S.C. § 102

Claim 1 was rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 5,532,432 to Peoples *et al.* ("Peoples"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiner asserts that Peoples teaches all of the limitation of claim 1. However, Peoples does not contemplate the production of any polyhydroxyhexanoate, because Peoples does not contemplate a PHB polymerase that accepts both 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl CoA, nor does Peoples contemplate providing the proper feedstock for such production.

Allowance of claims 1, 6, 7, 10 and 14-21 is respectfully solicited.

Respectfully submitted,



Todd S. Hofmeister
Reg. No. 53,029

Date: March 10, 2003

HOLLAND & KNIGHT LLP
One Atlantic Center, Suite 2000
1201 West Peachtree Street
Atlanta, Georgia 30309-3400
(404) 817-8473
(404) 817-8588 (Fax)

Marked Up Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. (Four times amended) A method for the biological production of polyhydroxyalkanoate containing 3-hydroxyhexanoate [comprising growing a transgenic] in *E. coli* [having at least one bacterial transgene encoding a PHA polymerase incorporating C_6 substrates and at least one enzyme selected from the group consisting of] expressing a phbA thiolase gene encoding an enzyme that converts butyryl-CoA and acetyl CoA to beta-ketohexanoyl-CoA, a phbB reductase gene that encodes an enzyme that converts beta-ketohexanoyl-CoA to beta-hydroxyhexanoyl-CoA, and a phbC polymerase gene that encodes an enzyme that polymerizes 3-hydroxybutyryl CoA, the improvement comprising expressing in the *E. coli* a D-specific enoyl-CoA hydratase and ß-hydroxyacyl-ACP-coenzymeA transferase, and providing feedstocks for the transgenic *E. coli*, wherein the enzymes are expressed in a sufficient amount to produce [production of] polyhydroxybutyrate-co- polyhydroxyhexanoate [by the transgenic *E. coli* occurs].

6. (Two times Amended) The method of claim 1 wherein the phbC polymerase gene encoding a PHA polymerase enzyme that incorporates C_6 substrates is incorporated into the bacterial chromosome.

7. (Twice amended) The method of claim 1 wherein the phbC polymerase gene is from a bacteria selected from the group consisting of *Aeromonas caviae, Comamonas testosteroni,*

Thiocapsia pfenigii, Chromatium vinosum, Bacillus cereus, Nocardia carolina, Nocardia salmonicolor, Rhodococcus ruber, Rhodcoccus rhodocrous, and *Rhodospirilum rubrum.*

Please cancel claims 8 and 9.

10. (Three times Amended) The method of claim [9] 1 wherein the [phaJ transgene] genes encoding the D-specific enoyl-CoA hydratase and ß-hydroxyacyl-ACP-coenzymeA transferase [is] are isolated from a bacterium selected from the group consisting of *R. eutropha, Klebsiella aerogenes, P. putida*, and *Aeromonas caviae.*

Please cancel claims 11-13.

14. (amended) The method of claim 11 wherein the [organism] *E. coli* expresses a broad range reductase that is active on C_6 substrates.

15. (twice amended) The method of claim 11 wherein the [organism] *E. coli* expresses a polymerase that accepts 3-hydroxyhexanoyl CoA and 3-hydroxybutyryl CoA.

16. (amended) The method of claim 11 wherein the [organism] *E. coli* expresses a thiolase accepting acetoacetyl CoA.

17. (amended) The method of claim 11 wherein the [organism] *E. coli* expresses an enzyme selected from the group consisting of thiolases specific for 3-ketohexanoyl CoA, reductase active on 3-ketohexanoyl CoA, and 3-hydroxyhexanoyl CoA.

18. (amended) The method of claim 8 wherein the [organism] *E. coli* expresses one or more fatty acid biosynthetic enzymes.

19. (amended) The method of claim 18 wherein the fatty acid biosynthetic enzymes [are

enzymes converting] <u>convert</u> acyl ACP to acyl CoA.

20. The method of claim 19 where the enzymes are selected from the group consisting of ACP-CoA transacylase, acyl ACP thioesterase, and acyl CoA synthase.

21. The method of claim 20 wherein the enzymes are acyl ACP thioesterase and acyl CoA synthase.

Please cancel claims 22-27 and 31.